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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PCT-7165			ent's file reference	FOR FURTHER A	CTION	See Notification	on of Transmittal of Internation camination Report (Form PC	onal CT/IPEA/416)
International application No. PCT/IT 03/00329				International filing date 28.05.2003	(day/mon	th/year)	Priority date (day/month/y 31.05.2002	rear)
C07	7D49	1/22	ent Classification (IPC) or bo					
SiG	SIGMA-TAU INDUSTRIE FARMACEUTICHE RIUNITE S.P.A							
1.	 This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36. 							
2.	2. This REPORT consists of a total of 10 sheets, including this cover sheet.							
	This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority						gs which have this Authority	
	(see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT). These annexes consist of a total of sheets.							
3.	This report contains indications relating to the following items:							
	1		Basis of the opinion					
	II ☐ Priority III ☐ Non-establishment of opinion with regard to		pinion with rogard to	povelty inventive etch and industrial applicability				
	IV 🖾 Lack of unity of invention		pinion with regard to novelty, inventive step and industrial applicability					
	V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability citations and explanations supporting such statement						applicability;	
	VI		Certain documents cite					
	VIII		Certain defects in the in					
	VIII Certain observations on the international application							
Date	Date of submission of the demand				Date of	completion of thi	ls report	
	10.12.2003					2004		
Name prelin	Name and mailing address of the international preliminary examining authority:				Authoriz	ed Officer		has Peterre
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465			Weisbr Telephor	rod, T ne No. +49 89 2	399-8931			

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/IT 03/00329

i.	Basis	of the	report
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1. With regard to the **elements** of the international application (Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)):

	De	scription, Pages							
	1-2	26	as originally filed						
	Cla	iims, Numbers							
	1-1	•	as originally filed						
2. With regard to the language , all the elements marked above were available or furnished to this Authorit language in which the international application was filed, unless otherwise indicated under this item.									
	The	These elements were available or furnished to this Authority in the following language: , which is:							
		the language of a tra	tion furnished for the purposes of the international search (under Rule 23.1(b)).						
			lication of the international application (under Rule 48.3(b)).						
		the language of a tra Rule 55.2 and/or 55.	anslation furnished for the purposes of international preliminary examination (under 3).						
3.	Wit inte	h regard to any nucl e rnational preliminary	eotide and/or amino acid sequence disclosed in the international application, the examination was carried out on the basis of the sequence listing:						
		contained in the inte	rnational application in written form.						
		filed together with th	e international application in computer readable form.						
			ntly to this Authority in written form.						
		furnished subsequer	ntly to this Authority in computer readable form.						
		The statement that to in the international a	he subsequently furnished written sequence listing does not go beyond the disclosure pplication as filed has been furnished.						
		The statement that the listing has been furnitude.	he information recorded in computer readable form is identical to the written sequence ished.						
4.	The	amendments have re	esulted in the cancellation of:						
		the description,	pages:						
		the claims,	Nos.:						
		the drawings,	sheets:						
5.		This report has been been considered to g	established as if (some of) the amendments had not been made, since they have so beyond the disclosure as filed (Rule 70.2(c)).						
		(Any replacement sh report.)	eet containing such amendments must be referred to under item 1 and annexed to this						
6.	Add	itional observations. i	f necessary:						

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/IT 03/00329

١V	•	Lack	(of	uni	ity	of	inv	rention
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١.	111 1	response to the invitation to restrict or pay additional fees, the applicant has:					
		restricted the claims.					
	\boxtimes	paid additional fees.					
		paid additional fees under pro	otest.				
		neither restricted nor paid add	ditional	fees.			
2.		This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.					
3.	This	This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is					
		complied with.					
		not complied with for the follow	wing re	easons:			
4.	Con	onsequently, the following parts of the international application were the subject of international preliminary camination in establishing this report:					
	\boxtimes	all parts.					
		the parts relating to claims No	s				
v.	Rea cita	easoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; itations and explanations supporting such statement					
1.	Stat	ement					
	Nov	eity (N)	Yes: No:	Claims Claims	2,4-6,9,10,13 1,3,7,8,11,12		
	Inve	ntive step (IS)	Yes: No:	Claims Claims	1-13		
	Indu	strial applicability (IA)	Yes: No:	Claims Claims	1-13		
2.	Cita	tions and explanations					

Form PCT/IPEA/409 (January 2004)

see separate sheet



International application No. PCT/IT 03/00329

Re Item I

Basis of the opinion

The application is directed to

- (i) camptothecin esters (I) (claims 1-4),
- (ii) process for preparing compounds (I) (independent claims 5 and 6),
- (iii) compounds (I) as medicaments,
- (iv) pharmaceutical compositions comprising compounds (I) (claims 8-10), and
- (v) the use of compounds (I) for the preparation of a medicament (claims 11-13).

Re Item IV

Lack of unity of invention

The application as filed is considered to lack unity of invention since its subject-matter relates not to one but rather to two separate inventions not linked together by a common underlying inventive concept as required by Rules 13.1 and 13.2 PCT.

The claims and inventions to which the separate inventions relate are grouped as follows (in the order chosen by the applicant).

- (1) Claims 1, 4, 7-13 (all part) and claims 2 and 6 (complete) directed to compounds
 (I) wherein n = m = 1, as well as subject matter referring to such compounds (I).
- (2) Claims 1, 4, 7-13 (all part) and claims 3 and 5 (complete) directed to compounds (I) wherein n = m = 0, as well as subject matter referring to such compounds (I).

The identified two inventions involve the technical feature of a "camptothecin-20-O-C(O)-A- moiety" as the sole common link. However, this feature cannot be accepted to constitute a special technical feature because it does not define a contribution over the prior art. The documents D1 and D2 disclose already water soluble camptothecin prodrugs respectively derivatives comprising such "camptothecin-20-O-C(O)-A- moiety". The document D1, in particular, discloses already certain present compounds (I) wherein A is methylene, n=m=0, and Y is -N*R₁₂R₁₃R₁₄ (D1, claim 1; and examples 1 and 2) as well as their therapeutic use in treating cancer. The document D2, furthermore, teaches already "camptothecin-20-O-C(O)-A-derivatives" with a 20-substituent of the formula -O-C(O)-(CH₂)₂-NH-C(O)-(CH₂)_m-NR¹⁰R¹¹ (cf. D2, claim 1, R⁷). Certain present compounds (I) (i.e. those wherein A is C₁₋₈ alkyl, n=m=1, and Y is C₁₋₈ alkyl-NR₁₂R₁₃; thus having a 20-substituent of the formula O-C(O)-C₁₋₈alkyl-C(O)-NH-C₁₋₈



8alkyl-NR12R13) differ from these compounds of D2 insofar as the amide group -(CO)n-(NH)_m- is retroinverted in comparison with the orientation of the amide group of the compounds of the prior art.

In view of the said prior art the problem underlying the present application is seen in the provision of further water soluble derivatives of camptothecin and the contributions claimed in the present application which are possibly made over the prior art are

- the provision of further water soluble camptothecin derivatives by retro-inverting the orientation of the amide group of the compounds of D2; and
- (b) the provision of further water soluble camptothecin derivatives by modifying e.g. the length of -A- or the nature of Y in the corresponding groups of the compounds of D1.

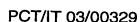
These contributions, however, have nothing more in common than each single of these contributions has in common with the prior art. Consequently, these contributions diverge in two different directions are, thus, not so linked as to form one single inventive concept, which would support the unity of the invention.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

- 1 Reference is made to the following documents.
 - D1: US-A-4 943 579, 24.07.1990.
 - D2: WO 01/49691 A, 12.07.2001.
 - D3: Matsumoto, H. et al. Bioorg. Med. Chem. Lett. 2001, 11, 605-609; cited in the application.
 - D4: Crossfire Beilstein, BRN's 7967940, 7563919; & Bioorg. Med. Chem. 1998, *6*, 551-562.
 - D5: Lerchen, H.-G.; von dem Bruch, K. J. Prakt. Chem. 2000, 342, 753-760.
 - D6: Crossfire Beilstein, BRN's 9312592, 9312810; & J. Med. Chem. 2003, 46, 190-193.

Document D6 was published after the priority date. Under the presumption that the priority is valid for the claimed matter the said document is not considered as



prior art under Rule 64.1 PCT.

- 2 Novelty
- In view of D1, D4, and D5 the application does not comply with the criterion of 2.1 novelty according to Article 33(2) PCT.

D1 discloses water soluble prodrugs of camptothecin according to the present formula (I) wherein R^1 to R^3 is hydrogen, A is methylene, n=m=0, and Y is - $N^{+}R_{12}R_{13}R_{14}$ (D1, claim 1, R = COCH₂NH₂ HCI, COCH₂NHCH₃ HCI, COCH₂NHC₂H₅ HCl, COCH₂N(C₂H₅)₂ HCl; and examples 1 and 2), their therapeutic use in treating cancer, and the corresponding pharmaceutical formulation (claims 2 and 3). The present claims 1, 3, 7, 8, 11, and 12 lack thus novelty in view of D1.

The document, furthermore, discloses the preparation of such compounds (I) comprising reacting camptothecin with chloroacetic anhydride (i.e. an activated carboxylic acid bearing a leaving group in the omega position) to camptothecin 20-O-chloroacetate, reacting the chloroacetate to the corresponding iodoacetate, and substituting the iodine leaving group with a primary or secondary amine to introduce the present Y group. The process according to present claim 5 appears thus merely formally novel over D1, because according to claim 5 the camptothecin is reacted with a carboxylic acid rather than with an activated derivative thereof.

D2 relates to camptothecin-20-O-beta-alanine esters of improved solubility and stability. In this context the document teaches camptothecin derivatives with a substituent in position 20 of the formula -O-C(O)-(CH₂)₂-NH-C(O)-(CH₂)_m-NR¹⁰R¹¹ (cf. D2, claim 1, R^7). Certain present compounds (I) (i.e. those wherein A is C_{1-8} alkyl, n = m = 1, and Y is C_{1-8} alkyl-NR₁₂R₁₃; thus having a 20-substituent of the formula O-C(O)-C₁₋₈alkyl-C(O)-NH-C₁₋₈alkyl-NR₁₂R₁₃) differ from these compounds of D2 insofar as the amide group $-(CO)_n-(NH)_m$ is retroinverted in comparison with the orientation of the amide group of the compounds of the prior art.

D3 relates to the design of water soluble prodrugs of sparingly water soluble drugs. The authors of the document exemplify their strategy with the prodrugs of an HIV protease inhibitor. These prodrugs comprise a prodrug moiety of the formula -O-C(O)-X-C(O)-NH-R (X is e.g. -CH $_2$ -CH $_2$ - and R is e.g. -(CH $_2$) $_2$ NH $_2$ HCI, -(CH₂)₂N(CH₃)₂ HCI, or -(CH₂)₂COOH), which is identical to the prodrug moiety of



the present compounds (I) wherein n=m=1. The document, however, is not relevant to the question of novelty of the present claimed matter, because the present camptothecin prodrugs (I) are not disclosed in the prior art document.

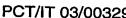
D4 and D5 disclose certain compounds (I) as synthesis intermediates wherein n = m = 0 and Y is -N⁺R₁₂R₁₃R₁₄ (D4, BRN's 7967940, 7563919; D5, page 754, compounds 4a-f; pages 757-759, compounds 4A-E). Present claims 1 and 3 lack thus novelty in view of these documents.

- 2.2 The document D6 discloses further present compounds (I) with n = m = 0 and $Y = -N^{+}R_{12}R_{13}R_{14}$ (BRN's 9312592, 9312810). The document may thus become relevant if the present claimed date of priority was invalid.
- 3 Inventive Step
- The application describes the synthesis of certain compounds (I) (pages 15-26, 3.1 examples 1-11) and shows that these compounds possess anticancer activity (pages 14-15). As can be understood from the application (pages 1-2, chapter "background of the invention"), these compounds (I) represent water soluble prodrugs of camptothecin, a topoisomerase I inhibitor of anticancer activity.
- 3.2 Invention (1) according to item IV above

D2 teaches already camptothecin derivatives as anticancer agents of improved solubility with a group -O-C(O)-(CH₂)₂-NH-C(O)-(CH₂)_m-NR¹⁰R¹¹ in position 20. Certain present compounds (I) bear a group -O-C(O)-C₁₋₈alkyl-C(O)-NH-C₁₋₈alkyl- $NR_{12}R_{13}$ in position 20 and differ from said compounds of D2 through the orientation of the amide group of the side-chain in position 20.

Starting from D2 as most relevant state of the art the problem underlying the aspect (1) of the application is seen in the provision of further water soluble derivatives of camptothecin.

The documents D3 relates to the design of water soluble prodrugs of sparingly water soluble drugs by utilizing a spontaneously cleavable linker strategy. For the design of such prodrugs, two auxiliary units, a solubilizing moiety and a selfcleavable spacer, are tandemly linked to the parent drug (cf. page 605. last paragraph; and page 606, figure 2). Combinations of such solubilizing and self-



cleavable moieties are e.g. represented by the formulae -O-C(O)-(CH₂)₂-C(O)-NH- CH_2 -COOH, -O-C(O)-(CH_2)₂-C(O)-NH-(CH_2)₂NH₂ HCl, or -O-C(O)-(CH_2)₂-C(O)-NH-(CH₂)₂N(CH₃)₂ HCI (cf. table 1) and are thus identical to the side-chain groups according to the aspect (1) of the present application. Although, the prodrug strategy of the document D3 has been exemplified with an HIV protease inhibitor, the document leaves no doubt that the general applicability of this approach is contemplated in the document D3 (cf. page 605. last paragraph; and page 606, figure 2).

As the pharmacophore of the present compounds and the compounds of D2 is represented by the camptothecin heterocycle itself (with lactone ring E being essential for cytotoxicity; the application page 1) and due to the very close structural similarity of the present compounds and the compounds of D2, it appears that the skilled person whishing to provide further camptothecin derivatives of the desired technical effect would, starting from D2 in combination with the general teaching of D3, consider the present claimed compounds (I) as obvious alternatives of the compounds of D2. Consequently, in the absence of any substantiated unexpected effect of the present claimed compounds (I) in comparison with the closest related compound(s) of D2 (i.e. the present compound wherein R^1 to $R^3 = H$, $A = -(CH_2)_2$ -, n = m = 1, and $Y = -(CH_2)_5$ - NH_3^+ CI^- ; in comparison with camptothecin-20-beta-Ala-Lys ester dihydrochloride according to page 16 of D2), no inventive step would be acknowledged.

Consequently the aspect (1) according to the present claims 1, 2, 4, 6, and 7-13 does, at present, not meet the requirements of Article 33(3) PCT.

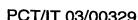
3.3 Invention (2) according to item IV above

Insofar as the aspect (2) of the application relates to novel compounds (I) the following observations would apply to the requirement of inventive step.

Certain novel compounds (I) according to the aspect (2) of the application differ from the compounds of D1 e.g. through the length of A as representing e.g. C_{2-8} alkylene instead of methylene. Further novel compounds (I) according to the aspect (2) of the application differ from the compounds of D1 either through R1 to R³ or through the nature of Y.

Starting from D1 as most relevant state of the art the problem underlying the

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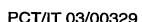
aspect (2) of the application is seen in the provision of further water soluble derivatives of camptothecin.

The document D2 shows already that compounds wherein A is longer than in the compounds of D1 (cf. D2, claim 1, $R^7 = -C(O)CH_2CH_2NR^8R^9$; i.e. $A = -CH_2CH_2$ instead of -CH2-) are compatible with the desired activity, and it is even stated that β -alanine esters of camptothecin have greater stability and solubility than esters of naturally occurring amino acids (cf. page 4). Consequently, starting from D1 in combination with D2, at least certain compounds (I) of the aspect (2) of the application appear to represent merely obvious alternatives of the compounds of D1, for which no inventive step would be acknowledged unless the applicant was able to substantiate an unexpected effect in comparison with the closest related compound(s) of D1 (e.g. the present compound (I) wherein $R^1-R^3=H$, n=m=0, A = $-CH_2CH_2$ -, Y = $-NH_3^+CI^-$ versus example 1 of D1; or the present compound (I) wherein $R^1-R^3=H$, n=m=0, $A=-CH_2CH_2$ -, $Y=-NHEt_2$ +Cl⁻ versus example 2 of D1). Consequently the aspect (2) according to the present claims 1, 3-5 and 7-13 does, at present, not meet the requirements of Article 33(3) PCT.

In this context it is also noted that for the requirement of unity to be met the subject-matter of the aspect (2) of the application should be characterized by a common distinguishing feature over the compounds of D1 which is at present not evident (cf. compounds (I) which differ from the compounds of D1 through A, through R1-R3, or through Y). Consequently, the aspect (2) of the application will, most probably, be divided into further non-unitary groups of inventions in the regional phase.

- 4 Deficiencies of the Application under Article 6 PCT
 - Claim 4 does not comply with Article 6 PCT for the following reasons.
- The chemical name of example 2 (identical with entry 1 of claim 4) does not 4.1 correspond with the product to be expected from the starting materials used in the preparation of example 2. This inconsistency between the experimental conditions ind the description and the compound for which protection is sought in the claim renders claim 4 unclear. In addition, the claimed compound is not comprised in the scope of claim 1 from which claim 4 is defined as dependent; this adds to the unclarity of the claim.





- 4.2 Furthermore, entry 3 of claim 4 (= example 4) does not fall under the scope of claim 1, thereby resulting in a lack of clarity of claim 4.
- 4.3 Finally, the name segments "benzylglicyl", "terbutylglycyl", and "2-methoxyphenylglycyl" in the entries 5-7, 9, and 10 of claim 4 leave the reader in doubt about the position of the benzyl, tert-butyl, and 2-methoxyphenyl substituents and add to the unclarity of the claim.
- Further Deficiencies of the Application 5

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in D1 and D2 is not mentioned in the description, nor are these documents identified therein.